DISTRIBUTION AND PROPERTIES OF INTRACELLULAR ALKALINE PHOSPHATASES[1]

EDITH JUDITH KRUGELIS[2]

Department of Zoology, Columbia University, New York

The chemical and structural composition and the interrelationships of the components of the cell have long been the concern of biologists, in hope of revealing the fundamental processes within the structural and functional unit of all living matter. The early studies of Miescher upon the chemical composition of the nucleus were contemporary with the studies of the structure of the nucleus by van Beneden, Flemming, and others, which later culminated in the firm establishment of the nucleus as the site of phenomena of central importance in the mechanism of Mendelian heredity. The biochemical line of investigation, started by Miescher, has had no such tradition of continuous progress as have the studies of chromosomal structure; but as a result of sporadic advances following the appearance of new methods and techniques, certain facts about the chemical composition of the cell and the nucleus have become firmly established. Miescher noted that the nucleus had a high content of organically bound phosphate; later researches (Levene and Bass, 1931) have fully confirmed this, and have led to a fairly comprehensive knowledge of the chemical composition of nucleic acid, the substance in which all this nuclear phosphate is contained. The unit of nucleic acid is the mononucleotide, a phosphoric acid ester of pentose sugar in glucosidic linkage with one of the purine or pyrimidine heterocyclic bases. The nucleic acids, which have been intensively studied, have been found to be polynucleotides with four different bases, and in their native form they are very highly polymerized, consisting in some cases of hundreds or thousands of nucleotides. There are two types of nucleic acid with respect to the sugar; one contains a pentose which whenever identified has been found to be d-ribose, and the other a desoxypentose found to be d-ribodesose. The wide application of the Feulgen cytochemical test for desoxypentose indicates that nucleic acid with this sugar does not occur outside of the chromatin of the cell nucleus (that is, in the chromosomes), and actual analysis of isolated nuclei and chromosomes has confirmed the view that desoxypentose is the characteristic nucleic acid of chromatin (Mirsky and Pollister, 1942). The name chromonucleic acid has recently been proposed (Pollister and Mirsky, 1943) for desoxypentose nucleic acid as a convenient way of emphasizing this striking limitation in distribution. Nucleic acids can also be located within the cell by means of the intense specific absorption of ultra-violet light by purine and pyrimidine bases, and Caspersson (1936, 1940) has made use of a combination of microspectroscopy and the Feulgen nucleal reaction to determine the distribution of the Feulgen negative pentose nucleic acids. This was found to be largely in the cell cytoplasm, the occurrence of pentose nucleic acid in the nucleus being confined to the plasmosome, or the non-chromatin nucleolus. The term plasmonucleic acid has been proposed to embody this information concerning the distribution of pentose nucleic acid. The nucleic acid makes up but a part, usually less than half, of the content of the nucleus. The remainder is apparently largely protein (Mirsky and Pollister, 1942). Much of this is of the basic type, either histone or protamine, but there is also evidence of protein of globulin type (Caspersson, 1936; Mazia and Jaeger, 1939).

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[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

[2] Present address: Department of Zoology, Vassar College, Poughkeepsie, N. Y.

In view of the fact that the nuclear chromatin contains a large amount of phosphate in desoxyribose nucleic acid, it is of considerable interest that definite evidence of phosphatase activity within chromatin has been found. Almost simultaneously the author (Krugelis, 1942) and Willmer (1942) reported the demonstration of alkaline phosphatase activity within chromosomes. This discovery stimulated the present study, the purpose of which is two-fold. The first purpose was to investigate the occurrence of alkaline phosphatase in all parts of the cell, and to investigate further the correlation of desoxyribose nucleic acid with alkaline phosphatase activity in the chromosomes. The material which seemed most promising for this first part of the study were the salivary gland chromosomes of Drosophila larvae, because of their peculiar structure which manifests itself in bands or regions rich in desoxyribose nucleic acid, alternating with interband regions poor or lacking in desoxyribose nucleic acid. This part of the investigation can be expressed in the form of a question: Is phosphatase activity located in the chromosomal regions rich in desoxyribose nucleic acid? The second part of the investigation followed from the first, and included the use as substrate of actual chemical substances that are found in the cell, in order to discover whether alkaline phosphatase is capable of hydrolyzing naturally-occurring substances with which its activity seems to be physically associated. If the activity of phosphatase is located in regions rich in desoxyribose nucleic acid, is it capable of hydrolyzing desoxyribose nucleic acid and other naturally occurring substances in the cell? This part of the investigation it was hoped would lead to some suggestion as to the possible role of phosphatase activity within the cell and the chromosome.

METHODS AND MATERIALS

The histochemical method used was that developed by Gomori (1939, 1941); when using smeared insect material it was found necessary to make some modifications of the preliminary steps. This process involved dissecting, and smearing the insect organs in isotonic saline, or Ringer's, made according to Buck and Melland (1942). After smearing, the material was fixed in 95 per cent ethyl alcohol vapors for one hour, then fixed in liquid 95 per cent ethyl alcohol for two hours. Material fixed as short a time as one hour and as long a time as 48 hours showed little appreciable difference in the results. The coverslips fell off readily in the alcohol, and the smears on the slides were washed briefly in distilled water and then incubated in the substrate solution. The mammalian organs used, namely, the kidney, liver, testis, intestine, and pancreas, were removed after ether anesthesia, fixed in 95 per cent ethyl alcohol for 24 hours, dehydrated in 100 per cent ethyl alcohol, cleared in benzene, and embedded in paraffin in the usual manner. Sections of 5 microns thickness were cut. To avoid any variation in mounting or incubating, sections of the five organs were mounted together on one slide. After deparaffinization, hydration, and washing, sections were ready for immersion and incubation in the substrate solution.

The process from this point on was the same for both the sectioned and the smeared materials. The substrate solution was of the following composition:

2 parts of 0.1 M sodium glycerophosphate (or 0.1 M other organic phosphate ester)
2 parts of 0.1 M calcium nitrate
1 part of 0.1 M veronal buffer at pH 9.4
5 parts of distilled water.

The pH was checked with thymol blue and adjusted to pH 9.0 to 9.2 with NaOH. The incubation was carried out at 25–28° C. and lasted from 12 to 24 hours. Following the incubation in the substrate, the slides containing the smears or the sections were immersed in 0.02 M calcium nitrate for one minute, then into 0.1 M cobaltous nitrate for two minutes, then dilute potassium sulfide for two minutes. After this visualization treatment, slides were washed, dehydrated, cleared, and mounted by the usual histological technique. The results were checked, in some cases, by parallel experiments in which the silver nitrate visualization method (Gomori, 1939) was used. When no activity was obtained in the substrate-incubated slides, the reaction was allowed to proceed for 72 hours to verify the negative results.

At the suggestion of Dr. A. E. Mirsky partially depolymerized nucleic acid was tried as the substrate. This depolymerization was accomplished by using desoxyribonuclease from beef pancreas, made according to the method of McCarty (1946). Desoxyribose nucleic acid is dissolved in distilled water and then placed in a nuclease medium of the following composition:

0.01 per cent magnesium sulfate
0.003 M gelatin
0.025 M veronal-HCl buffer at pH 7.5.

The nuclease is allowed to react upon the desoxyribose nucleic acid for 24 hours in which time the solution becomes clear and loses its viscosity. This change is used as a criterion for the depolymerization of the nucleic acid. This depolymerization leaves the nucleic acid in only a less highly polymerized state; and does not, for example, reduce it to an approximation of tetranucleotide. After the depolymerizing reaction, the solution is heated to 60° C. for fifteen minutes to destroy the activity of the nuclease on the desoxyribose nucleic acid, and then is used as the substrate source of phosphate ester.

The biological materials used in this study were insect and mammalian organs. The salivary glands of the larvae of Drosophila were used for the first part of the experimentation. The larvae were raised at 17–18° C. and at room temperature. The glands were dissected out when the larvae were full grown and had left the food prior to pupation. The species of Drosophila investigated included the Brazilian species *Drosophila pallidipennis* obtained through the generosity of Professor T. Dobzhansky, *D. simulans*, *D. virilis,* and *D. ananassae* obtained from the stocks at the Biological Laboratories, Cold Spring Harbor, N. Y. The major part of the investigation on Drosophila was done on *Drosophila melanogaster*.

The mammalian organs used were those of mice obtained from the stocks maintained at Columbia University by Professor L. C. Dunn and Dr. S. Gluecksohn-Schoenheimer. The testes were used either after being smeared in the body fluid surrounding the tubules, or after being fixed and embedded as the other organs were.

Yeast nucleic acid and adenylic acid were obtained from the Schwartz Laboratories, New York; thymonucleic (desoxyribose nucleic) acid and nuclease were generously supplied by Dr. A. E. Mirsky of the Rockefeller Institute for Medical Research; guanylic acid, sodium guanylate, and cytidylic acid were obtained from the Levene collection of chemicals at the Rockefeller Institute, also through the kindness of Dr. A. E. Mirsky. Photographic materials were provided from a grant to Columbia University by The Rockefeller Foundation.

Experimental Data

Intracellular occurrence and localization of alkaline phosphatase activity in the salivary gland cells of Drosophila melanogaster

Upon microscopic examination of smears of larval salivary glands of Drosophila, it is evident that in the preparations which have been incubated in the substrate, there is a much greater precipitate of cobaltous sulfide, indicating alkaline phosphatase activity, than in the smears incubated in control solutions. A diffuse precipitate occurs throughout the cytoplasm, but there is a much higher density within the nucleus (Fig. 1). This agrees with the observations of Moog (1944), on the tissues of the chick embryo that the nuclei were never less reactive than the cytoplasm and that the nuclei, unlike the cytoplasm, were never negative in reaction. Dounce (1943) using nuclei isolated from rat liver, also found that alkaline phosphatase activity is much greater in nuclei than in total liver.

Within the nucleus the precipitate is sharply localized in the chromosomes and nucleolus. The nuclei of the control slides show slight, if any, precipitate, with the exception that there is always some cobaltous sulfide in the control nucleoli (Fig. 2), but this is always considerably less in amount than that in nucleoli of substrate-incubated preparations.

The most dense precipitate is found within the chromosomes. When smeared in Ringer's solution, which is necessary to preserve the activity of the enzyme, the details of the chromosome structure are by no means as distinct as in the acetic acid smears that have been used in mapping bands. Nevertheless one can be certain that the cobaltous sulfide within the chromosome is concentrated in transverse bands, alternating with regions of very little or no precipitate (Figs. 3 and 5). This observation was reported earlier (Krugelis, 1945) and it has recently been confirmed by Danielli and Catcheside (1945) who compare the individual bands with specific regions of the cytogenetic chromosomal maps. In the preparations of Figures 3 and 5 the identity of the regions of phosphatase precipitate with the bands of acetic acid smears was established by a direct cytological observation. This method made use of the fact that the cobaltous sulfide precipitate is not altered by the standard Feulgen cytochemical procedure, that it becomes possible to superimpose the nucleal reaction on a phosphatase test. Figure 4 is from such a preparation; and comparison with Figure 5, the same chromosome after the phosphatase reaction but before the Feulgen test, shows that the bands in which the enzyme activity is localized correspond strictly with the Feulgen positive bands that form the basis of the familiar cytogenetic maps. The superposition of the second procedure merely changes the brown band to a purple brown, which is clearly a combination of cobaltous sulfide and basic fuchsin. This combination of the two cytochemical tests shows, in a vivid manner, that the highest level of alkaline phosphatase activity within the salivary gland cell occurs in exactly the region that contains a very high concentration of organic phosphate in the form of desoxyribose nucleic acid.

Properties and distribution of intracellular alkaline phosphatases

The occurrence of the enzyme activity and the desoxyribose nucleic acid within the same parts of the salivary gland chromosome suggests a functional relationship, which suggestion was put to test by using other phosphate esters as substrates for the enzyme activity. In order to elucidate a more specific picture of possible enzyme activity *in vivo* within the nucleus and the cell, esters were chosen which are normally present in the cell, and which the enzyme would consequently be expected to hydrolyze. The cellular substrates used, along with sodium glycerophosphate as the standard, were purine and pyrimidine nucleotides, ribose nucleic acid (presumably not highly polymerized), and desoxyribose nucleic acid (both polymerized and partially depolymerized). These substrates were tried on various mouse tissues for the presence of the enzyme activity in the cells. The testis was used for the chromosomal localization, since the other tissues contained nuclei which were mainly in the resting stages. In the results the black-brown precipitate of cobaltous sulfide indicates the site of alkaline phosphatase activity on the specific organic substrate in the incubating solution, and the density of the precipitate may be regarded as a measure of the intensity of the enzyme activity.

The photomicrographs (cf. Plates II, III, and IV) illustrate the results of these experiments; and roughly quantitative estimates of the intensities of the reaction are summarized in Table I. With sodium glycerophosphate and the nucleotides as substrates, the precipitate is not sharply localized, though it is always more dense in the nucleus and chromosomes than in the cytoplasm. By contrast, the reactions with depolymerized nucleic acids result in precipitates which are restricted to particular parts of the cell. On the slide in which depolymerized chromonucleic (desoxyribose nucleic) acid has been used as substrate the dark nuclei and chromosomes stand out very distinctly against a colorless cytoplasmic background, an appearance not unlike that of a Feulgen preparation. On the other hand, if plasmonucleic (ribose nucleic) acid is the substrate, the slide as a whole is intensely dark due to cytoplasmic precipitate, and the nuclei appear as light areas. The two types of slides compare with one another somewhat as do a photographic positive and negative. The chromonucleic acid precipitate is not uniformly distributed throughout the

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EXPLANATION OF PLATE I

The dark precipitate indicates the phosphatase activity. Photomicrographs made from smear preparations. Figures 1, 3–5 show phosphatase activity with sodium glycerophosphate as the substrate.

FIGURE 1. Alkaline phosphatase activity in salivary gland of *Drosophila melanogaster* larva. Magnification is 200 ×.

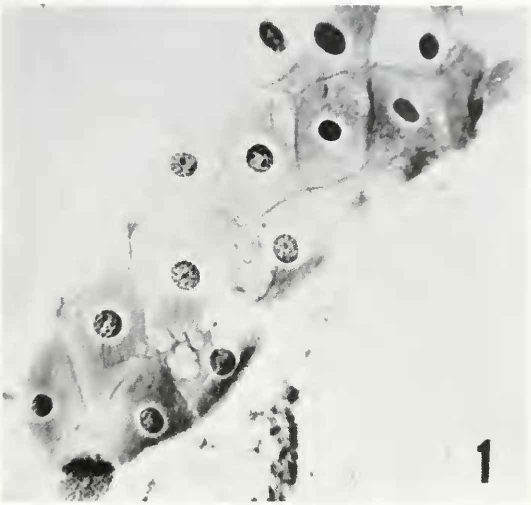
FIGURE 2. Salivary gland nucleus from control slide which had no substrate for the alkaline phosphatase activity. Magnification is 1000 ×.

FIGURE 3. Salivary gland nucleus showing alkaline phosphatase activity. Magnification is 1000 ×.

FIGURE 4. Salivary gland chromosomes with the Feulgen reaction applied over the phosphatase reaction precipitate. Magnification is 3000 ×.

FIGURE 5. Salivary gland chromosomes (same chromosomes as Fig. 4) showing alkaline phosphatase activity before Feulgen reaction applied over the precipitate. Magnification is 3000 ×.

Plate I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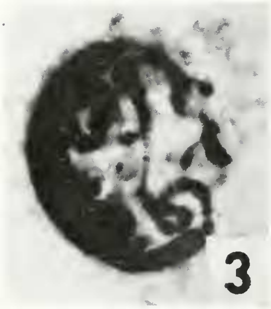
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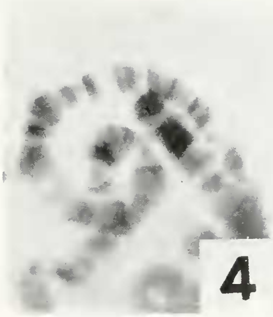
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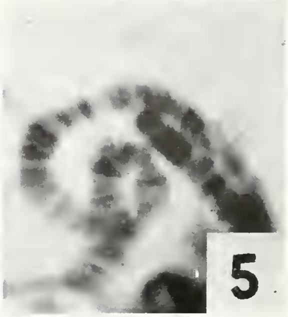
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nucleus. Instead it occurs only within the formed structures of the nucleus, the chromatin and the plasmosome nucleolus.

In an attempt to find enzyme differences involved in producing such specific localizations of alkaline phosphatase activity, these reactions were subjected to various conditions which might alter the reaction environment. Exposures to temperatures of 55° C. or over for a period of 5 minutes or longer produced complete irreversible inactivation of all the enzyme reactions. Magnesium ions in the concentration of 0.01 M magnesium sulfate showed some activation with the cytoplasmic reaction on ribose nucleic acid, and little or no increase on the nuclear and the general reactions. This very slight activating effect is in agreement with Schmidt and Thannhauser's (1943) observation that there is but slight effect of magnesium on intestinal alkaline phosphatase activity with sodium glycerophosphate as substrate.

TABLE I

Phosphatase reaction with different substrates

Substrate	Intestine		Testes		Liver		Pancreas		Kidney	
	c	n	c	n	c	n	c	n	c	n
Sodium glycerophosphate	1	2	1	2	0	2	±1	2	3	2
Adenylic acid, guanylic acid, cytidylic acid	1	2	1	2	0	2	0	2	1	2
Desoxyribose nucleic acid	0	0	0	0	0	0	0	0	0	0
Depolymerized desoxyribose nucleic acid	0	2	0	2	0	2	0	2	1	2
Ribose nucleic acid	2	±1	2	1	1	±1	2	±1	2	1

Density of cobaltous sulfide precipitate recorded from visual estimates. 0, no reaction; ± doubtful; 1, definite reaction; 2, strong reaction; 3, very strong reaction. Each record based on microscopic examination of at least 20 slides. Column c, cytoplasmic precipitate; column n, nuclear precipitate.

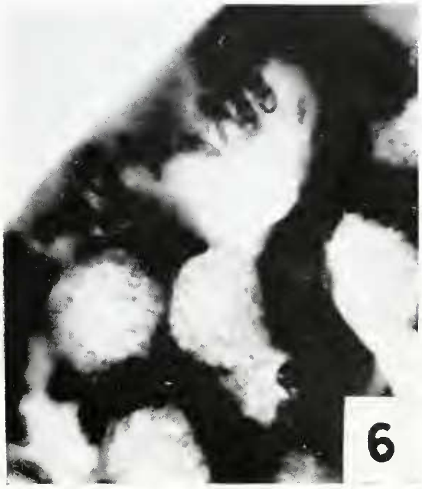
Cyanide ions in the concentration of 0.01 M potassium cyanide completely inhibited all three types of reaction.

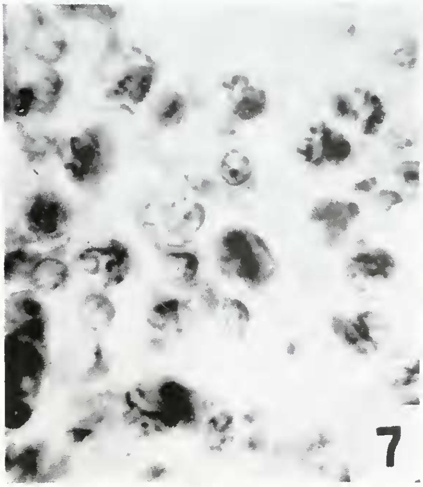
Arsenate ions in the concentration of 0.01 M sodium arsenate showed a more selective effect on the different reactions than did the environmental influences mentioned above. In the presence of arsenate, the alkaline phosphatase activity with the nucleic acids as substrates is completely suppressed. The glycerophosphate splitting, by contrast, in most cases proceeds at its normal rate in a medium which contains arsenate. A roughly quantitative estimation of the inhibition or suppression of activity by arsenate ions is presented in Table II.

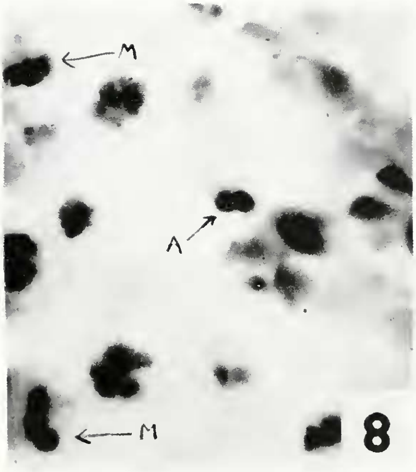
DISCUSSION

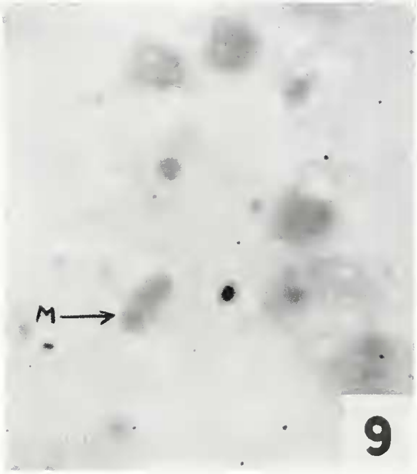
The differences in site of alkaline phosphatase activity in the same tissues when using different phosphate bearing substrates indicate localized reactions of three

PLATE II









Photomicrographs from 5 micra thick sections. Figures 6–8 show alkaline phosphatase activity with sodium glycerophosphate as substrate.

FIGURE 6. Small intestine of mouse showing activity of the enzyme distributed in the mucosa and submucosa layers. Magnification is 1000 ×.

FIGURE 7. Testis of mouse. Magnification is 1000 ×.

FIGURE 8. Testis of mouse. *M* indicates chromosomes in metaphase. *A* indicates chromosomes in anaphase. Magnification is 1000 ×.

FIGURE 9. Testis of mouse from a control slide with no substrate for enzyme activity applied. *M* indicates chromosomes in metaphase. Magnification is 1000 ×.

types, as follows: first, a general alkaline phosphatase reaction in both the cytoplasm and the nucleus, as in the case where sodium glycerophosphate and nucleotides are used as substrates; second, a definite nuclear reaction with little or no activity in the cytoplasm, as in the case of depolymerized desoxyribose nucleic acid as substrate; third, a definite cytoplasmic reaction with little reaction in the nucleus, as in the case of ribose nucleic acid as substrate. Since the enzymes cannot be isolated at this time, and since the nuclear and the cytoplasmic reactions are not absolutely specifically nuclear or cytoplasmic, they will be listed as reaction types with reference only to the location of the activity observed. With the different substrates used, the total reaction of phosphatase activity is somehow produced, and this is detected by the location of the cobaltous sulfide precipitate. The final reactions produced under a variety of substrate conditions must be due to at least three different enzymes, working in at least two different complexes.

TABLE II

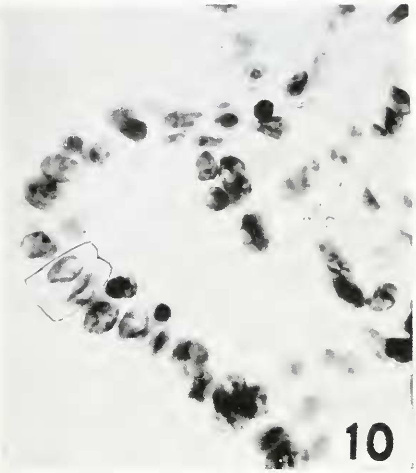
Intensity of precipitate of phosphatase reaction in different tissues using different substrates with and without arsenate ions

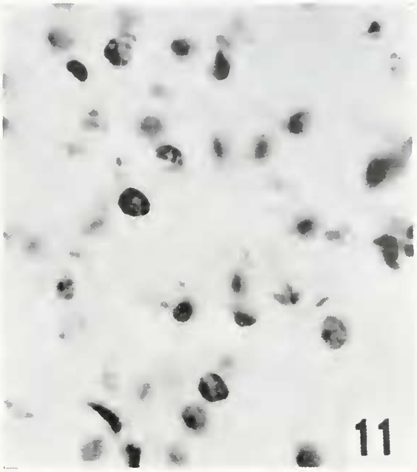
Substrate	Intestine		Testes		Liver		Pancreas		Kidney	
	c	n	c	n	c	n	c	n	c	n
Sodium glycerophosphate										
No arsenate ions. . . .	1	2	1	2	0	2	±1	2	3	2
With arsenate ions.	1	1*S1	1	2	0	0*S3	±1	2*S0	2	±1*S1
Depolymerized desoxyribose n. acid										
No arsenate ions	0	2	0	2	0	2	0	2	1	2
With arsenate ions.	0	0*S3	0	0*S3	0	0*S3	0	±1*S2	0	0*S3
Ribose nucleic acid										
No arsenate ions	2	±1	2	1	1	±1	2	±1	2	1
With arsenate ions. . . .	0	0*S3	2	0*S3	0	0*S3	0	0*S3	2	1*S0

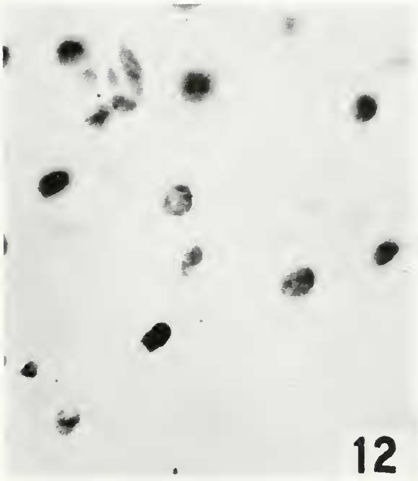
*S = suppression estimates.
S0 is no suppression.
S1 is slight suppression.
S2 is much suppression.
S3 is complete suppression.
Each record represents observation on 8 experimental slides.

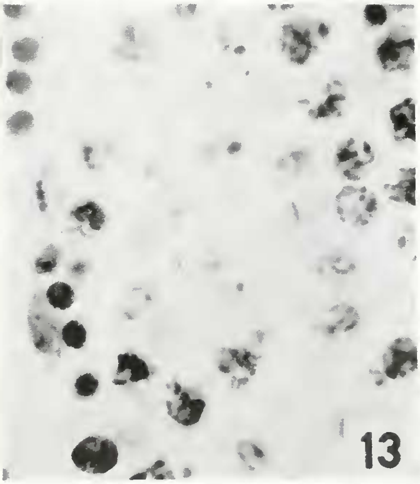
The suggestion that the reactions observed are due to complexes of enzymes is based on the chemical structure of nucleic acids. The ribose and desoxyribose nucleic acids, which were applied as substrates, are polymers of mononucleotides, which are considered to be linked to each other by an ester bond between the phosphate group of one nucleotide and the sugar group of the neighboring nucleotide, thus making the nucleic acids diesters of phosphoric acid all along the chain except in the terminal monoester of phosphoric acid. The total phosphatase action on the nucleic acids might be considered as essentially due to a specific desoxyribose nucleic acid phosphodiesterase, and a specific ribose nucleic acid phosphodiesterase action liberating mononucleotides of the nucleic acids, which then are hydrolyzed by a phospho-

PLATE III









Photomicrographs from 5 micra sections. Figures 10–13 show alkaline phosphatase activity with depolymerized desoxyribose nucleic acid as substrate.

FIGURE 10. Small intestine of mouse showing the distribution of the enzyme activity in the mucosa layer. Magnification is 1000 ×.

FIGURE 11. Kidney of mouse showing enzyme activity in the proximal tubules seen in cross-section. Magnification is 1000 ×.

FIGURE 12. Pancreas of mouse showing distribution of enzyme activity. Magnification is 1000 ×.

FIGURE 13. Testis of mouse showing enzyme activity. Magnification is 1000 ×.

monoesterase to liberate the inorganic phosphate. Thus the precipitate formed when the nucleic acids are applied as substrates is due to the activity of at least two different enzyme complexes, as follows: in the *cytoplasm*, first, a specific phosphodiesterase ("plasmonucleodiesterase") liberates ribonucleotides; second, phosphomonoesterase liberates inorganic phosphates from these nucleotides; in the *nucleus*, first, a specific phosphodiesterase[3] ("chromonucleodiesterase") liberates desoxyribosenucleotides; second, phosphomonesterase liberates inorganic phosphate from these nucleotides. This specificity of the diesterase follows from the fact that ribose nucleic acid will not serve as substrate for the nuclear diesterase activity, nor will desoxyribose nucleic acid serve for the cytoplasmic activity. With regard to the monoesterase activity, however, no such specificity has been detected, for the products of ribose nucleic acid hydrolysis serve equally well as substrates for phosphate production in either nucleus or cytoplasm.

While the action of the phosphodiesterase in freeing mononucleotides from lower polynucleotides (depolymerized nucleic acid) presumably can only occur by attack upon the linkage between the phosphate of one nucleotide and the sugar of the adjacent unit, it is evident that there is considerable restriction upon the exact nature of the bond which can be so attacked. If the enzyme were able to hydrolyze the bond at many points along a nucleic acid chain (consisting, let us say, of 2000 nucleotides) the diesterase should also function as a depolymerase, and a phosphate precipitate should be formed when the long, polynucleotide chains (polymerized nucleic acid) are used as substrate. Even after 72 hours action, however, there is no visible precipitate under these conditions, in contrast to the depolymerized nucleic acid experiments in which a dense precipitate is formed in a few hours. The type of bond which the diesterase can attack is evidently one which is enormously multiplied by a process of depolymerization. A most obvious view of enzyme specificity that would agree well with these facts is that the diesterase can attack only the bond between a terminal nucleotide and the penultimate nucleotide. Considering, for example, an extreme case, depolymerization of a 2000 unit polynucleotide chain to the minimum tetranucleotide should increase the number of bonds which such a specific diesterase can attack by a factor of 500. If specific terminal hydrolysis is the mechanism, the amount of mononucleotide that would become available by diesterase action upon the end of a highly polymerized nucleic acid chain would surely give an amount of phosphate precipitate so slight that it would be cytologically undetectable.

It is highly important to the question of the functional significance of the distribution of the phosphatases acting on the nucleic acids, that this actually coincides in a striking manner with the known locations of desoxyribose and ribose nucleic acids within the cell. The phosphatase activity on depolymerized desoxyribose nucleic acid is restricted to the chromatin of the nucleus, the only part of the cell in which this type of nucleic acid is found. By contrast, the ribose nucleic acid phosphatase activity is in the cytoplasm, a region in which only ribose nucleic acid has ever been demonstrated. (The only marked discrepancy is the occurrence of desoxyribose

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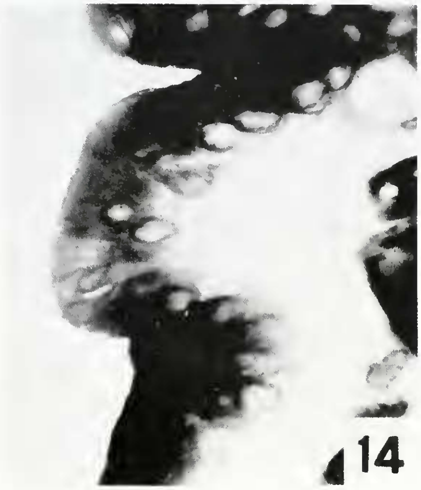
[3] Concerning the existence of this nuclear diesterase, there is some possible supporting evidence from the work by Mazia and Ballentine, reported by Mazia (1941), on an intranuclear enzyme from Arbacia eggs. Their enzyme, termed polynucleotidase, was active at a pH 9.0 and was capable of reacting on desoxyribose nucleic acid still in a polymerized form.

Plate IV



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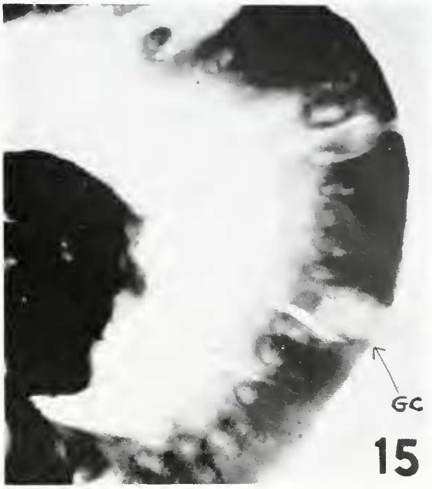

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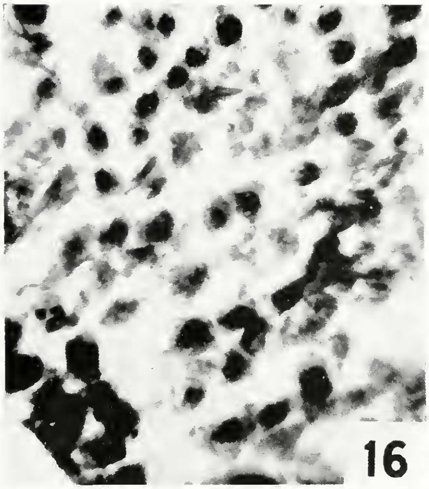

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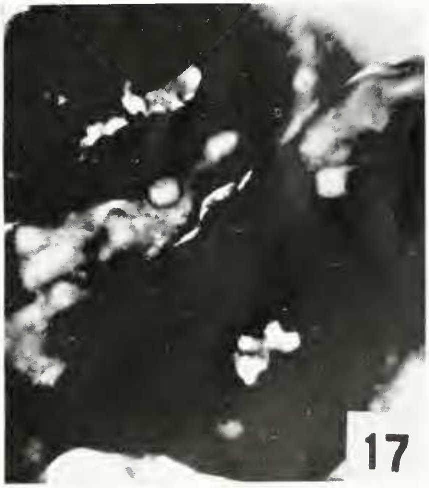

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Photomicrographs from 5 micra thick sections. Figures 14–17 show alkaline phosphatase activity with ribose nucleic acid as the substrate.

Figure 14. Small intestine of mouse showing the enzyme activity in the cells of the mucosa layer. Magnification is 1000 ×.

Figure 15. Small intestine of mouse showing goblet cells in the mucosa layer and the distribution of the enzyme activity. Magnification is 1000 ×.

Figure 16. Testis of mouse showing the enzyme activity. A denser precipitate is found in the nuclei of testis cells than in nuclei of other tissues under the conditions of the same substrate. Magnification is 1000 ×.

Figure 17. Kidney of mouse, showing enzyme activity in the proximal tubules in cross-section. Magnification is 1000 ×.

nucleic acid phosphatase activity in the nucleolus, which, since it is Feulgen negative, is considered to contain ribose nucleic acid.)[1]

Certain possible *in vivo* functions of these alkaline phosphatases are at once obvious. Not only can the nuclear diesterase split off terminal mononucleotides, as in these experiments, but in the reverse direction, it may conceivably catalyze the terminal growth in the development of nucleotide chains. One may easily picture the later stages of synthesis of a full length nucleic acid chain as involving the cooperation of two enzymes: the diesterase slowly builds up short chains by terminal growth and this is followed by the action of the depolymerase type of enzyme catalyzing the union of these short chains into the long complex which is such an important structural component of a chromosome.

While it is also obvious that catalysis of the synthesis of a mononucleotide from a nucleoside by phosphomonesterase is an essential step in nucleic acid synthesis, one's attention here tends rather to focus on the possibilities of dephosphorylation of nucleotide as a source of energy for nuclear and cytoplasmic reactions. Thus energy for synthesis of chromosomes and their products may or may not be available according to whether the nucleotides are structurally isolated from phosphomonesterase activity by being bound in nucleic acid chains, or whether as a result of a successive action of nuclear depolymerase, and "chromonucleodiesterase," there is mononucleotide available for dephosphorylation. Similarly the actual availability of energy for such cyclic nuclear mechanical processes as chromosome coiling and mitotic movement may be dependent upon a cycle of binding and release of mononucleotide from its nucleic acid storehouse.

SUMMARY

1. Using the histochemical test for alkaline phosphatase reaction in the larval salivary glands of several species of Drosophila, activity was found to be present in three main parts of the cell; the cytoplasm, the nucleolus, and the chromosomes.

2. Phosphatase activity was found rather generally distributed in both the cytoplasm and the nucleus. Within the larval salivary gland chromosomes, the enzyme activity was localized in those chromosomal regions which are Feulgen positive, and thus corresponds to the regions containing large concentrations of desoxyribose nucleic acid.

3. Different naturally occurring phosphate bearing substances were used as phosphatase substrates on mouse tissues, and resulted in demonstration of three different types of phosphatase reactions based on the localization of the enzyme activity.

a) A general reaction with phosphatase activity located in both nucleus (nucleolus and chromosomes) and the cytoplasm was present when sodium glycerophosphate and nucleotides were used as substrates.

b) No phosphatase reaction occurred on polymerized desoxyribose nucleic acid, but a specific nuclear reaction (nucleolus and chromosomes) was present when nuclease-depolymerized desoxyribose nucleic acid was used as a substrate.

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[1] We do not actually know the location of the possible substrates for the diesterase activity, since the methods for localizing nucleic acids do not, in all likelihood, preserve any but the high polymers, and it may well be that the lower polymers of the sort used as substrates are not the same in distribution as the larger complexes. In such a difference may lie the explanation of the discrepancies above.

c) A strong cytoplasmic reaction with slight reaction in the nucleus was present when ribose nucleic acid was used as a substrate.

4. Subjection to several environmental variables produced little further evidence as to the differences among these three types of localized reactions.

5. The three types of alkaline phosphatase reactions observed were suggested to be due to at least two phosphodiesterases and a phosphomonesterase.

6. The nuclear phosphatase complex and the cytoplasmic phosphatase complex each probably consist of a specific phosphodiesterase, which splits the ester linkage between the phosphate of one nucleotide and the sugar of the neighboring nucleotide, and a phosphomonesterase which splits the second ester linkage and liberates inorganic phosphate.

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